IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Hodge, Martin
Serial No.: 09/965,313
Filed: September 26, 2001
For: NOVEL IL9/IL2 RECEPTOR_LIKE MOLECULES AND USES THEREFOR

Group Art Unit: 1644
Examiner: NOT YET ASSIGNED

Assistant Commissioner for Patents
Washington, D.C. 20231

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December 4, 2001
Date of Signature and of Mail Deposit

Kerri Pollard Schray

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above referenced application, please amend the specification as follows:

At page 6, line 29 through page 7, line 5, please replace the paragraph with the following:

Figure 1A and 1B shows the amino acid sequence alignment for the human and murine 16445 proteins (SEQ ID NO:2 and SEQ ID NO:4, respectively) encoded by human 16445 (SEQ ID NO:1) and its murine orthologue 16445 (SEQ ID NO:3) with the human IL-2 receptor beta chain (hIL-2Rb; SP Accession Number P14784; SEQ ID NO:5), murine IL-2 receptor beta chain (mIL-2Rb; SP Accession Number P16297; SEQ ID NO:6), human IL-9 receptor (hIL-9R; SP Accession Number Q01113; SEQ ID NO:7), and murine IL-9 receptor (mIL-9R; SP Accession Number Q01114; SEQ ID NO:8). The sequence alignment was generated using the Clustal method.

At page 13, lines 12 through 17, please replace the paragraph with the following:

The h16445 protein displays similarity to the human IL-2 receptor beta chain (SEQ ID NO:5; approximately 36.9% identity over a 130 amino acid overlap), the murine IL-2 receptor

beta chain (SEQ ID NO:6; 32.7% identity over a 110 amino acid overlap), the human IL-9 receptor (SEQ ID NO:7; approximately 29.7% identity over a 158 amino acid overlap), and the murine IL-9 receptor (SEQ ID NO:8; approximately 28.3% identity over a 166 amino acid overlap) (see Figure 1A and 1B).

At page 13, line 25 through page 14, line 2, please replace the paragraph with the following:

The murine clone, m16445, encodes an approximately 2.5 Kb mRNA transcript having the corresponding cDNA set forth in SEQ ID NO:3. This transcript has a 1587 nucleotide open reading frame (nucleotides 391-1976 of SEQ ID NO:3), which encodes a 529 amino acid protein (SEQ ID NO:4) having a molecular weight of approximately 58.3 kDa. An analysis of the full-length m16445 polypeptide predicts that the N-terminal 19 amino acids represent a signal peptide. This polypeptide represents the protein sequence encoded by the murine orthologue of the h16445 gene. The mouse 16445 protein shares approximately 64.4% identity with the human 16445 protein disclosed in SEQ ID NO:2 (see Figure 1A and 1B).

At page 77, lines 4-25, please replace the two paragraphs with the following paragraphs:

The identified clone h16445 encodes a transcript of approximately 2.3 Kb (corresponding cDNA set forth in SEQ ID NO:1). The open reading frame (nt 349-1965) of this transcript encodes a predicted 538 amino acid protein (SEQ ID NO:2) having a molecular weight of approximately 59.1 kDa. A search of the nucleotide and protein databases revealed that h16445 encodes a precursor polypeptide that shares similarity with several cytokine receptor proteins. An alignment of the protein sequences having highest similarity to the h16445 precursor polypeptide is shown in Figure 1A and 1B. The alignment was generated using the Clustal method with PAM250 residue weight table and sequence identities were determined by FASTA (Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448).

The h16445 protein displays similarity to the human IL-2 receptor beta chain (approximately 36.9% over a 130 amino acid overlap; SEQ ID NO:5; SP Accession Number P14784; Hatakeyama *et al.* (1989) *Science* 244(4904):551-556). It also displays similarity to the murine IL-2 receptor beta chain (approximately 32.7% identity over a 110 amino acid overlap; SEQ ID NO:6; SP Accession Number P16297; Kono *et al.* (1990) *Proc. Natl. Acad. Sci. USA*

87(5):1806-1810); the human IL-9 receptor (approximately 29.7% identity over a 158 amino acid overlap; SEQ ID NO:7; SP Accession Number Q01113; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694; Chang *et al.* (1994) *Blood* 83(11):3199-3205; Kermouni *et al.* (1995) *Genomics* 29(2):371-382); (approximately 28.3% identity over a 166 amino acid overlap) to the murine IL-9 receptor (SEQ ID NO:8; SP Accession Number Q01114; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694) (see Figure 1A and 1B).

REMARKS

This Preliminary Amendment amends paragraphs of the specification to correctly reflect Figure identifications, specifically to refer to Figure 1 correctly as Figure 1A and 1B. Submitted concurrently herewith are Appendix A, Clean Copy of Amendments to Specification and Appendix B, Version to Show Changes Made to Specification.

Replacement pages 6, 7, 13, 14, and 33, are filed herewith with Response to Notice to File Corrected Application Papers solely to reflect the correct Figure identification. No new matter is entered by virtue of the amendments. Entry of the amendments made herein is respectfully requested.

December 4, 2001

Respectfully submitted,

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Appendix B

Version to Show Changes Made to Specification

At page 6, line 29 through page 7, line 5, please replace the paragraph with the following:

Figure 1A and 1B shows the amino acid sequence alignment for the human and murine 16445 proteins (SEQ ID NO:2 and SEQ ID NO:4, respectively) encoded by human 16445 (SEQ ID NO:1) and its murine orthologue 16445 (SEQ ID NO:3) with the human IL-2 receptor beta chain (hIL-2Rb; SP Accession Number P14784; SEQ ID NO:5), murine IL-2 receptor beta chain (mIL-2Rb; SP Accession Number P16297; SEQ ID NO:6), human IL-9 receptor (hIL-9R; SP Accession Number Q01113; SEQ ID NO:7), and murine IL-9 receptor (mIL-9R; SP Accession Number Q01114; SEQ ID NO:8). The sequence alignment was generated using the Clustal method.

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The murine clone, m16445, encodes an approximately 2.5 Kb mRNA transcript having the corresponding cDNA set forth in SEQ ID NO:3. This transcript has a 1587 nucleotide open reading frame (nucleotides 391-1976 of SEQ ID NO:3), which encodes a 529 amino acid protein (SEQ ID NO:4) having a molecular weight of approximately 58.3 kDa. An analysis of the full-length m16445 polypeptide predicts that the N-terminal 19 amino acids represent a signal peptide. This polypeptide represents the protein sequence encoded by the murine orthologue of the h16445 gene. The mouse 16445 protein shares approximately 64.4% identity with the human 16445 protein disclosed in SEQ ID NO:2 (see Figure 1A and 1B).

At page 77, lines 4-25, please replace the two paragraphs with the following paragraphs:

The identified clone h16445 encodes a transcript of approximately 2.3 Kb (corresponding cDNA set forth in SEQ ID NO:1). The open reading frame (nt 349-1965) of this transcript encodes a predicted 538 amino acid protein (SEQ ID NO:2) having a molecular weight of approximately 59.1 kDa. A search of the nucleotide and protein databases revealed that h16445 encodes a precursor polypeptide that shares similarity with several cytokine receptor proteins. An alignment of the protein sequences having highest similarity to the h16445 precursor polypeptide is shown in Figure 1A and 1B. The alignment was generated using the Clustal method with PAM250 residue weight table and sequence identities were determined by FASTA (Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448).

The h16445 protein displays similarity to the human IL-2 receptor beta chain (approximately 36.9% over a 130 amino acid overlap; SEQ ID NO:5; SP Accession Number P14784; Hatakeyama *et al.* (1989) *Science* 244(4904):551-556). It also displays similarity to the murine IL-2 receptor beta chain (approximately 32.7% identity over a 110 amino acid overlap; SEQ ID NO:6; SP Accession Number P16297; Kono *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87(5):1806-1810); the human IL-9 receptor (approximately 29.7% identity over a 158 amino acid overlap; SEQ ID NO:7; SP Accession Number Q01113; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694; Chang *et al.* (1994) *Blood* 83(11):3199-3205; Kermouni *et al.* (1995) *Genomics* 29(2):371-382); (approximately 28.3% identity over a 166 amino acid overlap) to the murine IL-9 receptor (SEQ ID NO:8; SP Accession Number Q01114; Renauld *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89(12):5690-5694) (see Figure 1A and 1B).

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant IL-9/IL-2 receptor-like protein activity or nucleic acid expression by administering an agent that is an IL-9/IL-2 receptor-like modulator to the subject. In one embodiment, the IL-9/IL-2 receptor-like modulator is an IL-9/IL-2 receptor-like protein. In another embodiment, the IL-9/IL-2 receptor-like modulator is an IL-9/IL-2 receptor-like nucleic acid molecule. In other embodiments, the IL-9/IL-2 receptor-like modulator is a peptide, peptidomimetic, or other small molecule.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic lesion or mutation characterized by at least one of the following: (1) aberrant modification or mutation of a gene encoding an IL-9/IL-2 receptor-like protein; (2) misregulation of a gene encoding an IL-9/IL-2 receptor-like protein; and (3) aberrant post-translational modification of an IL-9/IL-2 receptor-like protein, wherein a wild-type form of the gene encodes a protein with an IL-9/IL-2 receptor-like activity.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of an IL-9/IL-2 receptor-like protein. In general, such methods entail measuring a biological activity of an IL-9/IL-2 receptor-like protein in the presence and absence of a test compound and identifying those compounds that alter the activity of the IL-9/IL-2 receptor-like protein.

The invention also features methods for identifying a compound that modulates the expression of IL-9/IL-2 receptor-like genes by measuring the expression of the IL-9/IL-2 receptor-like sequences in the presence and absence of the compound.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A and 1B shows the amino acid sequence alignment for the human and murine 16445 proteins (SEQ ID NO:2 and SEQ ID NO:4, respectively) encoded by human 16445 (SEQ ID NO:1) and its murine orthologue 16445 (SEQ ID NO:3) with the

human IL-2 receptor beta chain (hIL-2Rb; SP Accession Number P14784; SEQ ID NO:5), murine IL-2 receptor beta chain (mIL-2Rb; SP Accession Number P16297; SEQ ID NO:6), human IL-9 receptor (hIL-9R; SP Accession Number Q01113; SEQ ID NO:7), and murine IL-9 receptor (mIL-9R; SP Accession Number Q01114; SEQ ID NO:8). The sequence alignment was generated using the Clustal method.

The human and murine 16445 protein sequences share approximately 64.4% identity as determined by pairwise alignment. The h16445 protein shares approximately 36.9% identity over a 130 amino acid overlap with the human IL-2 receptor beta chain, approximately 32.7% identity over a 110 amino acid overlap with the murine IL-2 receptor beta chain, approximately 29.7% identity over a 158 amino acid overlap with the human IL-9 receptor, and approximately 28.3% identity over a 166 amino acid overlap with the murine IL-9 receptor, as determined by FASTA.

Figure 2 shows expression of h16445 in various tissues and cell types relative to expression in human hepatoma cell line Hep3B.

Figure 3A and 3B shows the effect of the ectopic expression of h16445 on cytokine-induced CAT expression in cells transfected with Type I cytokine receptors.

Figure 4 shows the increase in IL-9 induced CAT expression mediated by an IL-9R/h16445 cytoplasmic-domain receptor chimera.

Figure 5 shows FACs analysis screening of hybridoma supernatants (clone #s 4, 7, or 8) for binding to: (A) the GPI-linked h16445 extracellular domain expressed by HEK293 cells transiently transfected with a plasmid encoding amino acids 1-234 of the extracellular domain of h16445 plus a His tag and the C-terminal signal sequence from human placental alkaline phosphatase (GPI-linker signal), or (B) the h16445 extracellular domain expressed by HEK293 cells transiently transfected with a plasmid encoding the full-length h16445 (B, C, and D). Relative fluorescence intensity exhibited by these transfected cells tagged with particular hybridoma supernatants (represented by peak 2 in panels A-D) is shown versus that exhibited by untransfected cells (represented by peak 1 in panels A-D), which served as the control.

Figure 6 shows FACs analysis screening of h16445-specific hybridoma supernatants (clone #s 3, 4, or 27) for binding to human tonsil CD19+ cells. Expression of h16445 as detected by staining with the h16445-specific hybridoma supernatants (peak

receptor-like protein possesses a fibronectin type III domain, from aa 120-215, and a U-PAR/Ly-6 domain, from aa 230-255, as predicted by HMMer, Version 2. The fibronectin type III domain is one of three types of internal repeats within the plasma protein fibronectin. The tenth fibronectin type III repeat contains an RGD cell recognition sequence in a flexible loop between two strands. Type III modules are present in both extracellular and intracellular proteins. See, for example, Petersen *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:137-141. The U-PAR/Ly-6 domain is a urokinase plasminogen activator surface receptor involved in binding urokinase plasminogen activator. This domain is responsible for signal transduction and is found in the family of Ly-6 T-cell antigens. See, for example, Behrendt *et al.* (1991) *J. Biol. Chem.* 266:7842-7847, and Ploug *et al.* (1993) *J. Biol. Chem.* 268:17539-17546.

The h16445 protein displays similarity to the human IL-2 receptor beta chain (SEQ ID NO:5; approximately 36.9% identity over a 130 amino acid overlap), the murine IL-2 receptor beta chain (SEQ ID NO:6; 32.7% identity over a 110 amino acid overlap), the human IL-9 receptor (SEQ ID NO:7; approximately 29.7% identity over a 158 amino acid overlap), and the murine IL-9 receptor (SEQ ID NO:8; approximately 28.3% identity over a 166 amino acid overlap) (see Figure 1A and 1B).

A plasmid containing the h16445 cDNA insert was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia, on _____, and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. ə 112.

The murine clone, m16445, encodes an approximately 2.5 Kb mRNA transcript having the corresponding cDNA set forth in SEQ ID NO:3. This transcript has a 1587 nucleotide open reading frame (nucleotides 391-1976 of SEQ ID NO:3), which encodes a 529 amino acid protein (SEQ ID NO:4) having a molecular weight of approximately 58.3 kDa. An analysis of the full-length m16445 polypeptide predicts that the N-terminal 19 amino acids represent a signal peptide. This polypeptide represents the protein sequence encoded by the murine orthologue of the h16445 gene. The mouse 16445 protein shares

approximately 64.4% identity with the human 16445 protein disclosed in SEQ ID NO:2 (see Figure 1A and 1B).

An analysis of the disclosed m16445 polypeptide sequence (SEQ ID NO:4) using the MEMSAT program predicts transmembrane segments from amino acids (aa) 7-23 and 415-434. Transmembrane segments were also predicted from aa 219-235 and 396-415 of the presumed mature peptide sequence. Prosite program analysis was also used to predict various sites within the m16445 protein sequence. N-glycosylation sites were predicted at 73-76, 97-100, 104-107, 125-128, and 182-185. A glycosaminoglycan attachment site was predicted from aa 430-433. Protein kinase C phosphorylation sites were predicted at aa 117-119, 131-133, and 209-211. Casein kinase II phosphorylation sites were predicted at aa 19-22, 50-53, 140-143, 213-216, 299-302, 378-381, 391-394, 442-445, 472-475, and 498-501. A tyrosine kinase phosphorylation site was predicted at 153-160. N-myristoylation sites were predicted at 16-21, 355-360, 427-432, 433-438, 466-471, 477-482, and 493-498. A growth factor and cytokine receptor signature 2 sequence was predicted at aa 212-218. Analysis with HMMer, Version 2, predicted a fibronectin type III domain from aa 120-215 in the mouse IL-9/IL-2 receptor-like protein, similar to that described for the human 16445 protein. A FN3_2 domain from aa 120-209 was also predicted for this protein by HMMer analysis.

The IL-9/IL-2 receptor-like sequences of the invention are members of a family of molecules (the "Type 1 cytokine receptor family") having conserved functional features. The term "family" when referring to the proteins and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of murine origin and a homologue of that protein of human origin, as well as a second, distinct protein of human origin and a murine homologue of that protein. Members of a family may also have common functional characteristics.

Preferred IL-9/IL-2 receptor-like polypeptides of the present invention have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or 4. The term "sufficiently identical" is used herein to refer to a first amino acid or

sequence determination and analysis, which revealed a more extensive reading frame with further similarity to known cytokine receptors. Upon further analysis, the clone h16445 was identified.

The identified clone h16445 encodes a transcript of approximately 2.3 Kb (corresponding cDNA set forth in SEQ ID NO:1). The open reading frame (nt 349-1965) of this transcript encodes a predicted 538 amino acid protein (SEQ ID NO:2) having a molecular weight of approximately 59.1 kDa. A search of the nucleotide and protein databases revealed that h16445 encodes a precursor polypeptide that shares similarity with several cytokine receptor proteins. An alignment of the protein sequences having highest similarity to the h16445 precursor polypeptide is shown in Figure 1A and 1B. The alignment was generated using the Clustal method with PAM250 residue weight table and sequence identities were determined by FASTA (Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448).

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Using a similar database mining strategy, clone m16445 was identified from a three-week-old murine LTBMC (long-term bone marrow cell) library made by stimulating the cells with heat-inactivated yeast hyphae at a hyphae:cell ratio of 2.3:1. The identified clone m16445 encodes a transcript of approximately 2.5 Kb (corresponding cDNA set forth in SEQ ID NO:3). The open reading frame (nt 391-1976) of this transcript encodes a predicted 529 amino acid protein (SEQ ID NO:4) having a